ATTACHMENT A

Product Category

Categories contain either sub-categories or products. The icon will display a description about the category (not all categories have descriptions). The category name hyperlink will display more sub-categories or products for that category. When products are displayed, click on the catalog number hyperlink to view the products detail page.

All Categories » Novagen » Competent Cells/Media » Strains for Protein Expression » T7 Expression Host Strain Competent Cells

Cat. No.	Product Name	Brand
69041	B834(DE3) Competent Cells	Novagen
69042	B834(DE3)pLysS Competent Cells	Novagen
69450	BL21(DE3) Competent Cells	Novagen
69451	BL21(DE3)pLysS Competent Cells	Novagen
69053	BLR(DE3) Competent Cells	Novagen
69956	BLR(DE3)pLysS Competent Cells	Novagen
69453	HMS174(DE3) Competent Cells	Novagen
69454	HMS174(DE3)pLysS Competent Cells	Novagen
69284	NovaBlue(DE3) Competent Cells	Novagen
71345	Origami™ 2(DE3) Competent Cells	Novagen
71346	Origami™ 2(DE3)pLysS Competent Cells	Novagen
70837	Origami™ B(DE3) Competent Cells	Novagen
70839	Origami™ B(DE3)pLysS Competent Cells	Novagen
70627	Origami™(DE3) Competent Cells	Novagen
71397	Rosetta™ 2(DE3) Competent Cells	Novagen
71403	Rosetta™ 2(DE3)pLysS Competent Cells	Novagen
70954	Rosetta™(DE3) Competent Cells	Novagen
70956	Rosetta™(DE3)pLysS Competent Cells	Novagen
71351	Rosetta-gami™ 2(DE3) Competent Cells *	Novagen
71352	Rosetta-gami™ 2(DE3)pLysS Competent Cells	Novagen
71136	Rosetta-gami B(DE3) Competent Cells	Novagen
71137	Rosetta-gami B(DE3)pLysS Competent Cells	Novagen
71055	Rosetta-gami™(DE3) Competent Cells	Novagen
71057	Rosetta-gami™(DE3)pLysS Competent Cells	Novagen
71059	RosettaBlue™(DE3) Competent Cells	Novagen
71034	RosettaBlue™(DE3)pLysS Competent Cells	Novagen
70623	Tuner™(DE3) Competent Cells	Novagen
70624	Tuner™(DE3)pLysS Competent Cells	Novagen

Competent *E. coli* for all your Cloning and Expression needs

Invitrogen™
life technologies

Comparative information for competent cells

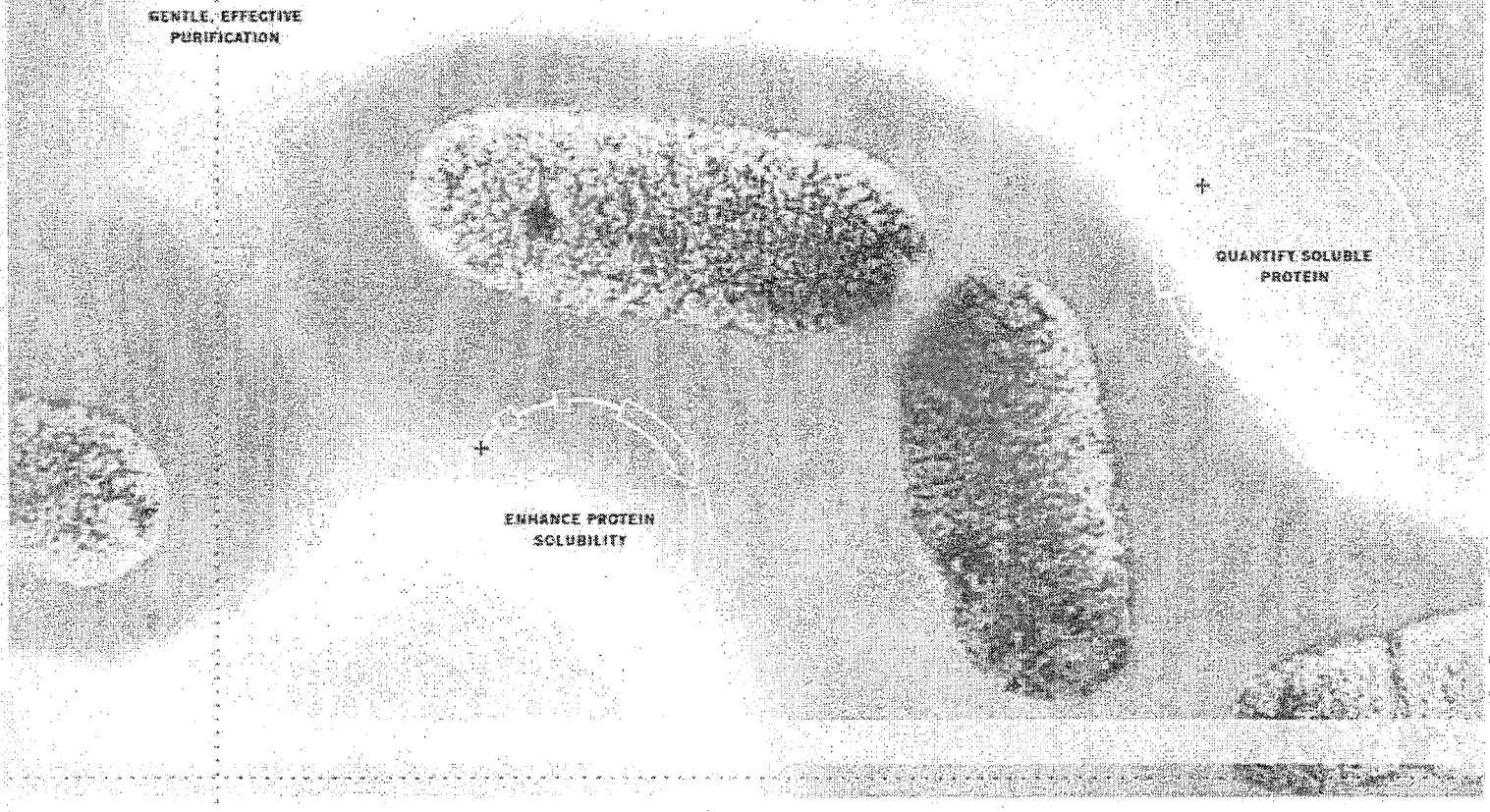
Strains	Cat. no.	Quantity	Efficiency*	Key Features: Reduces recombination events (recA)	Improves mini-prep DNA plasmid quality (endA)	Blue/white screening (lacZΔM15)	Cloning methylated DNA (mcrA, mcrB, mrr)	F' Episome (for ss DNA production)	For transformation of large plasmid DNA
Routine Cloning									
new One Shot® OmniMAX™ T1 Phage-Resistant [a]	C8520-03	20 x 50 µl	> 5 x 10⁹	•	•	•	•	•	•
One Shot® TOP10	C4040-10	10 x 50 µl	> 1 x 10⁹	•	•	•	•	—	—
One Shot® TOP10	C4040-03	20 x 50 µl	> 1 x 10⁹	•	•	•	•	—	—
One Shot® TOP10	C4040-06	40 x 50 µl	> 1 x 10⁹	•	•	•	•	—	—
One Shot® MAX Efficiency® DH10B™ T1 Phage-Resistant [a]	12331-013	20 x 50 µl	> 1 x 10⁹	•	•	•	•	—	—
One Shot® MAX Efficiency® DH5α™ T1 Phage-Resistant [a]	12297-016	20 x 50 µl	> 1 x 10⁹	•	•	•	—	—	—
MAX Efficiency® DH10B™	18297-010	5 x 200 µl	> 1 x 10⁹	•	•	•	•	—	—
MAX Efficiency® DH5α™ T1 Phage-Resistant [a]	12034-013	5 x 200 µl	> 1 x 10⁹	•	•	•	—	—	—
MAX Efficiency® DH5α™	18258-012	5 x 200 µl	> 1 x 10⁹	•	•	•	—	—	—
One Shot® TOP10F'	C3030-03	20 x 50 µl	> 1 x 10⁹	•	•	•	•	•	—
One Shot® TOP10F'	C3030-06	40 x 50 µl	> 1 x 10⁹	•	•	•	•	•	—
One Shot® TOP10/P3 [f]	C5050-03	20 x 50 µl	> 1 x 10⁹	•	•	•	•	—	—
One Shot® INVαF'	C2020-03	20 x 50 µl	> 1 x 10⁸	•	•	•	•	•	—
One Shot® INVαF'	C2020-06	40 x 50 µl	> 1 x 10⁸	•	•	•	•	•	—
Library Efficiency® DH5α™	18263-012	5 x 200 µl	> 1 x 10⁸	•	•	•	—	—	—
Subcloning Efficiency™ DH5α™	18265-017	4 x 500 µl	> 1 x 10⁶	•	•	•	—	—	—
MC1061/P3 Ultracomp™ [f]	C663-03	5 x 300 µl	> 1 x 10⁸	—	—	—	•	—	—
Fast Growth									
new One Shot® Mach1™ T1 Phage-Resistant [a]	C8620-03	20 x 50 µl	> 1 x 10⁹	•	•	•			
new MultiShot™ StripWell Mach1™ T1 Phage-Resistant [a]	C8696-01	1 plate	> 1 x 10⁸	•	•	•			
High-Throughput Cloning									
MultiShot™ StripWell OmniMAX™ T1 Phage-Resistant [a]	C8596-01	1 plate	> 1 x 10⁸	•	•	•	•	•	•
MultiShot™ StripWell Mach1™ T1 Phage-Resistant	C8696-01	1 plate	> 1 x 10⁸	•		•			
Recombinant Protein Expression									
BL21-AI™ One Shot®	C6070-03	20 x 50 µl	> 1 x 10⁸	—	—	—	—	—	—
BL21 Star™(DE3) One Shot®	C6010-03	20 x 50 µl	> 1 x 10⁸	—	—	—	—	—	—
BL21 Star™(DE3)pLysS One Shot®	C6020-03	20 x 50 µl	> 1 x 10⁸	—	—	—	—	—	—
One Shot® BL21(DE3)	C6000-03	20 x 50 µl	> 1 x 10⁸	—	—	—	—	—	—
One Shot® BL21(DE3)pLysS	C6060-10	10 x 50 µl	> 1 x 10⁸	—	—	—	—	—	—
One Shot® BL21(DE3)pLysS	C6060-03	20 x 50 µl	> 1 x 10⁸	—	—	—	—	—	—
One Shot® BL21(DE3)pLysE	C6565-03	20 x 50 µl	> 1 x 10⁷	—	—	—	—	—	—
MAX Efficiency® DH5αF'IQ™	18288-019	5 x 200 µl	> 1 x 10⁸	•	•	• [b]	—	•	—
cDNA or Genomic Library Construction Using Chemically Competent Cells									
new One Shot® OmniMAX™ T1 Phage-Resistant [a]	C8520-03	20 x 50 µl	> 5 x 10⁹	•	•	•	•	•	•
new One Shot® MAX Efficiency® DH10B™ T1 Phage-Resistant [a]	12331-013	20 x 50 µl	> 1 x 10⁹	•	•	•	•	—	—

a *tonA* confers resistance to T1 and T5 phages
b Requires IPTG for blue/white screening
c *mcrB*, *mrr*
d GeneHogs® have been qualified with both a 7 kb and 150 kb BAC construct. Designed for cloning large constructs, BACs and PACs
e see **www.invitrogen.com/gateway** for more information
f For use with *supF* containing plasmids
* Transformants/µg pUC19 DNA

PROTEIN FUNCTION & ANALYSIS

Bacterial Protein Expression & Analysis

+ How do you solve protein expression problems?



STRATAGENE

E. coli Hosts That Overcome Expression Problems

E. coli expression systems are often your first choice because they are fast, simple, and provide extremely high yields. However, sometimes *E. coli* expression fails. To solve this problem, we offer innovative competent cells that dramatically improve *E. coli* as an expression host.

BL21-CodonPlus® Cells

Recombinant protein expression in *E. coli* can be difficult because codons that are rare in *E. coli* may be used more frequently by other organisms. Common symptoms of this problem – called codon bias – include low or nonexistent protein synthesis, early termination, and misincorporation of amino acids in the expressed protein. To solve this problem, we created the BL21-CodonPlus® RIPL strain[2,3,4] which contains extra copies of the *E. coli argU, ileY, leuW* and *proL* tRNA genes. These strains can be used to overcome expression problems from both AT- and GC-rich genomes (Figure 4). The original BL21-CodonPlus-RIL and -RP strains are optimized for AT- and GC-rich genomes respectively.

BL21-Gold Expression and Cloning Strain Saves Time

When codon bias is not a concern, we recommend cloning directly in the BL21-Gold strain[4]. This strain lacks the EndA 1 nuclease, an enzyme that rapidly degrades miniprep DNA. Cloning directly in the BL21-Gold strain saves you two days of work which would otherwise be spent on sub-cloning procedures in another *endA*- strain (Figure 5). This strain also carries the Hte phenotype[4] increasing the transformation efficiency 100-fold over the parental BL21 strain.

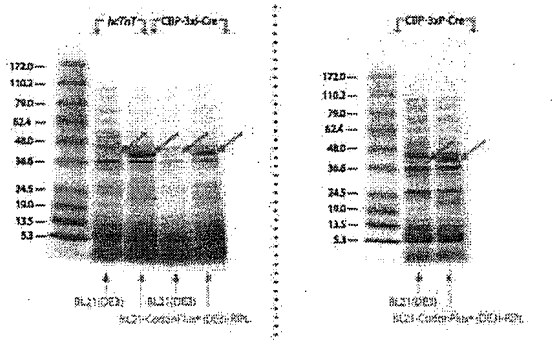


Figure 4
SUPERIOR EXPRESSION OF GENOMES WITH CODON BIAS
We expressed three genes whose expression is dependent on the expression of rare codons in either BL21(DE3) cells or BL21-CodonPlus® (DE3)-RIPL competent cells. BL21-CodonPlus® (DE3)-RIPL cells dramatically improve expression of proteins by overcoming codon bias compared to parental BL21(DE3) cells.

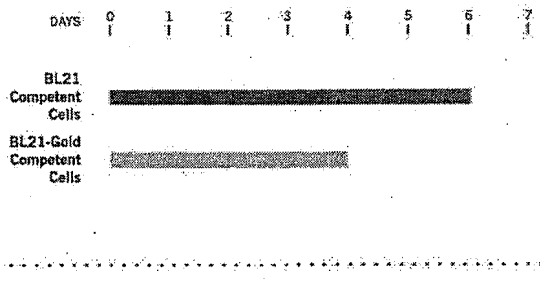


Figure 5
DIRECT CLONING IN BL21-GOLD CELLS SAVES TWO DAYS

VariFlex™ Triple-Tag Systems and Vector Sets			
N-TERMINAL SBP-SET-Q EXPRESSION SYSTEM	SBP SET Q	20 µg pBEn-SBP-SET1-Q in 3 reading frames 20 µg pBEn-SBP-SET2-Q in 3 reading frames 20 µg pBEn-SBP-SET3-Q in 3 reading frames 10 x 0.1 ml BL21-Gold (DE3) LacZ- competent cells 1.25 ml streptavidin resin 100 assays Q-tag detection reagents	240167
C-TERMINAL SBP-SET-Q EXPRESSION SYSTEM	SBP SET Q	20 µg pBEc-SBP-SET1-Q 20 µg pBEc-SBP-SET2-Q 20 µg pBEc-SBP-SET3-Q 10 x 0.1 ml BL21-Gold (DE3) LacZ- competent cells 1.25 ml streptavidin resin 100 assays Q-tag detection reagents	240179
N-TERMINAL SBP-SET-Q VECTOR SET	SBP SET Q	20 µg pBEn-SBP-SET1-Q in 3 reading frames 20 µg pBEn-SBP-SET2-Q in 3 reading frames 20 µg pBEn-SBP-SET3-Q in 3 reading frames	240166
C-TERMINAL SBP-SET-Q VECTOR SET	SBP SET Q	20 µg pBEc-SBP-SET1-Q 20 µg pBEc-SBP-SET2-Q 20 µg pBEc-SBP-SET3-Q	240178

Purification and Detection Reagents		
STREPTAVIDIN RESIN	1.25 ml	240105
VARIFLEX™ Q-TAG DETECTION REAGENTS	100 assays	240186
VARIFLEX™ BL21-GOLD LACZ- COMPETENT CELLS	10 x 0.1 ml	230135

Expression Hosts		
BL21-CODONPLUS® (DE3)-RIPL COMPETENT CELLS	10 x 0.1 ml	230280
BL21-CODONPLUS® RIL COMPETENT CELLS	10 x 0.1 ml	230240
BL21-CODONPLUS® RP COMPETENT CELLS	10 x 0.1 ml	230250
BL21-CODONPLUS® (DE3)-RIL COMPETENT CELLS	10 x 0.1 ml	230245
BL21-GOLD CELLS	10 x 0.1 ml	230130
BL21-GOLD (DE3) CELLS	10 x 0.1 ml	230132
BL21-GOLD (DE3) pLysS CELLS	10 x 0.1 ml	230134

Mutagenesis Kits		
QUIKCHANGE® II SITE-DIRECTED MUTAGENESIS KIT	10 reactions	200523
QUIKCHANGE® II SITE-DIRECTED MUTAGENESIS KIT	30 reactions	200524
QUIKCHANGE® II XL SITE-DIRECTED MUTAGENESIS KIT	10 reactions	200521
QUIKCHANGE® II XL SITE-DIRECTED MUTAGENESIS KIT	30 reactions	200522
QUIKCHANGE® MULTI SITE-DIRECTED MUTAGENESIS KIT	Academic Version, 30 reactions	200514
QUIKCHANGE® MULTI SITE-DIRECTED MUTAGENESIS KIT	Commercial Version, 30 reactions	200513

Protein Expression Tools		
STRATASCRIPT™ FIRST STRAND cDNA SYNTHESIS KIT	50 reactions	200420
STRATASCRIPT™ ONE-TUBE RT-PCR SYSTEM	50 reactions	600168
STRATASCRIPT™ TWO-TUBE RT-PCR SYSTEM	50 reactions	600170
STRATACLEAN™ RESIN	3 ml	400714
STRATACLEAN™ RESIN	9 ml	400715

LEGAL LANGUAGE

1: U.S. Patent No. 4,952,496. For academic or non-profit laboratories, an assurance letter accompanies the sale of the products. For commercial laboratories, a research use license agreement must be entered into prior to purchase of the products.

2: Patent Pending

3: Purchase of this product is accompanied by a limited use license to use the product as a reagent for research purposes. If Purchaser intends to use the product outside the scope of the license, he needs to enter into a License Agreement with F. Hoffmann-La Roche Ltd, Grenzacherstrasse 124, 4070 Basel, Switzerland or Hoffmann-La Roche Inc.

4: U.S. Patent No. 6,706,526

5: U.S. Patent Nos. 6,713,285, 6,391,548, 5,789,166 and 5,932,419 and patents pending

6: U.S. Patent Nos. 6,489,150, 6,444,428, 6,379,953, 6,333,165, 6,183,997, 5,948,663, 5,866,395, 5,556,772, 5,545,552 and patents pending

7: U.S. Patent Nos. 6,706,526, 5,707,841 and 5,512,468 and patents pending and equivalent foreign patents

8: Patents Pending
Use of the QuikChange® Multi Site-Directed Mutagenesis Kit, catalog # 200514, by commercial entities requires a commercial license from Stratagene. The QuikChange® Multi Site-Directed Mutagenesis Kit, catalog # 200513, is offered for sale to commercial entities with a limited use license.

9: Purchase of these products is accompanied by a license to use them in the Polymerase Chain Reaction (PCR) process in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or as purchased, i.e., an authorized thermal cycler.

10: Patents Pending

11: Use of these products for certain applications may require licenses from third parties in certain countries.

12: U.S. Patent No. 4,923,978

RELATED INFORMATION

- New Products
- Unit Size Increases
- FAQs for Competent Cells
- Technical Reference for Competent Cells

FAVORITE TOOLS

- Enzyme Finder
- NEBcutter
- NEBuffer Chart
- Double Digest Finder
- Isoschizomers
- DNA Sequences and Maps
- REBASE

SPECIAL OFFERS

Competent Cells

E. coli Cloning Strains

dam$^-$/*dcm*$^-$ Competent *E. coli*
NEB 10-beta Competent *E. coli* (High Efficiency)
NEB 10-beta Electrocompetent *E. coli*
NEB 5-alpha Competent *E. coli* (High Efficiency)
NEB 5-alpha Competent *E. coli* (Subcloning Efficiency)
NEB 5-alpha Electrocompetent *E. coli*
NEB 5-alpha F$'$*I*^{q} Competent *E. coli* (High Efficiency)
NEB Turbo Competent *E. coli* (High Efficiency)
NEB Turbo Electrocompetent *E. coli*

K.lactis Strains

K. lactis GG799 Competent Cells

E. coli Protein Expression Strains

NEB Express *I*^{q} Competent *E. coli* (High Efficiency)
NEB Express Competent *E. coli* (High Efficiency)
T7 Express *I*^{q} Competent *E. coli* (High Efficiency)
T7 Express *lysY/I*^{q} Competent *E. coli* (High Efficiency)
T7 Express *lysY* Competent *E. coli* (High Efficiency)
T7 Express Competent *E. coli* (High Efficiency)
T7 Express Crystal Competent *E. coli* (High Efficiency)
T7 Express High Efficiency Sampler

Special Offer

Phusion™ Site-Directed Mutagenesis Kit with NEB Turbo Competent *E. coli*

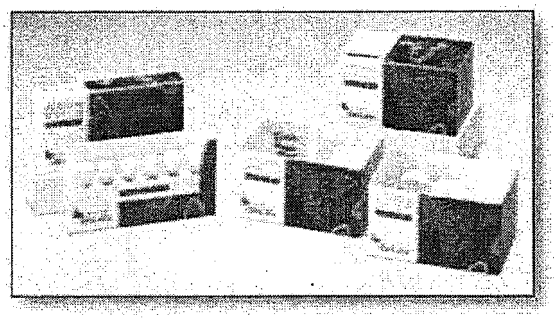
New England Biolabs, Inc.
Tel: 800-632-5227 (orders)
Tel: 800-632-7799 (support)
Fax: 978-921-1350
info@neb.com
www.neb.com

Competent Cells from NEB

NEB is pleased to offer several strains of optimized electrocompetent and chemically competent cells for cloning and protein expression.

Cloning Strains

NEB 5-alpha is a high efficiency derivative of DH5α™, the industry standard cloning strain. It is also offered in a *lacI*^{q} version for the cloning of toxic genes. NEB Turbo brings unmatchable speed to your transformations with visible colonies after just 6.5 hours. Other cloning strains include NEB 10-beta, a derivative of DH10B™, an excellent strain for transforming large plasmids and BACs, as well as *dam*$^-$/*dcm*$^-$, a strain for dam and dcm methylation free plasmid growth.



Protein Expression Strains

Try our protein expression strains for an extra level of confidence. NEB Express is an enhanced BL21 derivative available with or without the added control of IPTG induced expression of non-T7 plasmids from *lacI*^{q}. Several NEB strains feature the *lysY* gene for exceptional control of expression. LysY is a variant of T7 lysozyme lacking amidase activity making the cells less susceptible to lysis during induction, while retaining the ability to inhibit T7 RNA polymerase. Basal expression of the target gene is minimized without inhibiting IPTG-induced expression. *LysY* is encoded on a single-copy miniF plasmid that does not require antibiotic selection for propagation. T7 Express (an enhanced derivative of BL21, (DE3)) is available with or without the added control of *lacI*^{q}, and both versions can be purchased with or without the *lysY* feature. T7 Express *lysY/I*^{q} provides the highest level of uninduced control. T7 Express Crystal is a *metB* strain optimized for crystallographic experiments.

Convenient Formats

For your convenience, we offer all of these strains in two formats; 20 single-use transformation tubes or 6 tubes containing 200 µl each. Both formats are supplied with SOC outgrowth media and a pUC19 plasmid control. The most popular cloning strain, NEB 5-alpha is offered at a subcloning efficiency for substantial value. NEB 5-alpha, NEB 10-beta and NEB Turbo are also available in electrocompetent formats. See www.neb.com for protocols and tips on enhancing transformation efficiencies.

Advantages

- Extremely high efficiencies
- T1 phage resistance (*fhuA2*)
- Outgrowth media and control plasmid included
- A variety of convenient formats including single-use transformation tubes and, on request, 96 well formats
- Quality assurance – NEB scientists have been using these strains in house for over 20 years
- Bulk sales capabilities with custom packaging formats
- Free of animal products

Strain Selection Chart

Fastest Growth – Colonies Visible After 6.5 Hours	NEB Turbo Competent *E. coli**
Versatile Cloning	NEB 5-alpha Competent *E. coli**
Cloning of Toxic Genes	NEB 5-alpha F′ *I*^{q} Competent *E. coli*
Cloning of Large Plasmids and BACs	NEB 10-beta Competent *E. coli**
Growth of Unmethylated Plasmids	*dam*$^-$/*dcm*$^-$ Competent *E. coli*
Most Popular Non-T7 Expression Strain	NEB Express Competent *E. coli*
Control of IPTG Induced Expression	NEB Express *I*^{q} Competent *E. coli*
Most Popular T7 Expression Strain	T7 Express Competent *E. coli*
Reduced Basal Expression	T7 Express *I*^{q} Competent *E. coli*
Tight Control of Protein Expression by Inhibition of T7 RNA Polymerase	T7 Express *lysY* Competent *E. coli*
Highest Level of Expression Control	T7 Express *lysY/I*^{q} Competent *E. coli*
For Crystallography Experiments/SeMet Labeling	T7 Express Crystal Competent *E. coli*

**Also available as electrocompetent cells.*

(See other side)

Competent Cells from NEB (*continued*)

Strain Properties Table

Transformation Efficiency (cfu/µg)*	1-3 x 10^9	1-3 x 10^9	1-3 x 10^9	1-3 x 10^9	1-3 x 10^8	0.6-1 x 10^8	0.6-1 x 10^9	0.6-1 x 10^9	0.6-1 x 10^9	0.6-1 x 10^8	0.6-1 x 10^8	0.6-1 x 10^8
Strain	K12	K12	K12	K12	K12	B	B	B	B	B	B	B
T1 Phage Resistant	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Blue/White Screening	✓	✓	✓	✓	–	–	–	–	–	–	–	–
*lacI*q*	✓	–	✓	–	–	–	✓	–	✓	–	✓	–
lysY	–	–	–	–	–	–	–	–	–	✓	✓	–
Colonies Visible after 6.5 hours	✓	–	–	–	–	–	–	–	–	–	–	–
Endonuclease I Deficient	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Protease Deficient	–	–	–	–	–	✓	✓	✓	✓	✓	✓	✓
Eukaryotic DNA Cloning	–	–	–	✓	–	✓	✓	✓	✓	✓	✓	✓
M13 Phage Capable (F')	✓	–	✓	–	–	–	–	–	–	–	–	–
RecA Deficient	–	✓	✓	✓	–	–	–	–	–	–	–	–

*Transformation Efficiencies given are for high efficiency chemically competent strains. TE for electrocompetent strains is 1-4 x 10^{10} cfu/µg.

Cloning Strains

NEB Turbo Competent *E. coli*
Fastest growth – colonies visible after 6.5 hours

NEB Turbo Competent *E. coli* (High Efficiency)
C2984H 20 tubes x 0.05 ml.....$215.00
C2984I 6 tubes x 0.2 ml.....$165.00

NEB Turbo Electrocompetent *E. coli*
C2986K 6 tubes x 0.1 ml.....$200.00

NEB 10-beta Competent *E. coli*
Clone large plasmids and BACs

NEB 10-beta Competent *E. coli* (High Efficiency)
C3019H 20 tubes x 0.05 ml.....$170.00
C3019I 6 tubes x 0.2 ml.....$130.00

NEB 10-beta Electrocompetent *E. coli*
C3020K 6 tubes x 0.1 ml.....$160.00

NEB 5-alpha Competent *E. coli*
Versatile cloning strain

NEB 5-alpha Competent *E. coli* (High Efficiency)
C2987H 20 tubes x 0.05 ml.....$160.00
C2987I 6 tubes x 0.2 ml.....$125.00

NEB 5-alpha Competent *E. coli*
(Subcloning Efficiency)
C2988J 6 tubes x 0.4 ml.....$55.00

NEB 5-alpha Electrocompetent *E. coli*
C2989K 6 tubes x 0.1 ml.....$150.00

NEB 5-alpha F´ *I*q Competent *E. coli*
Clone toxic genes

NEB 5-alpha F´ *I*q Competent *E. coli*
(High Efficiency)
C2992H 20 tubes x 0.05 ml.....$160.00
C2992I 6 tubes x 0.2 ml.....$125.00

dam*-/*dcm*- Competent *E. coli
Grow plasmids free of dam and dcm methylation

dam-/*dcm*- Competent *E. coli*
C2925H 20 tubes x 0.05 ml.....$200.00
C2925I 6 tubes x 0.2 ml.....$155.00

Transformation Protocol

SOC Outgrowth Medium
B9020S 100 ml.....$55.00

Protein Expression Strains

NEB Express Competent *E. coli*
Most popular expression strain

NEB Express Competent *E. coli*
(High Efficiency)
C2523H 20 tubes x 0.05 ml.....$160.00
C2523I 6 tubes x 0.2 ml.....$125.00

NEB Express *I*q Competent *E. coli*
Control of IPTG-induced protein expression

NEB Express *I*q Competent *E. coli*
(High Efficiency)
C3037H 20 tubes x 0.05 ml.....$160.00
C3037I 6 tubes x 0.2 ml.....$125.00

T7 Express Competent *E. coli*
Most popular T7 expression strain

T7 Express Competent *E. coli* (High Efficiency)
C2566H 20 tubes x 0.05 ml.....$160.00
C2566I 6 tubes x 0.2 ml.....$125.00

T7 Express *I*q Competent *E. coli*
Reduced basal expression

T7 Express *I*q Competent *E. coli*
(High Efficiency)
C3016H 20 tubes x 0.05 ml.....$160.00
C3016I 6 tubes x 0.2 ml.....$125.00

T7 Express *lysY* Competent *E. coli*
Tight control by inhibition of T7 RNA Pol

T7 Express *lysY* Competent *E. coli*
(High Efficiency)
C3010H 20 tubes x 0.05 ml.....$160.00
C3010I 6 tubes x 0.2 ml.....$125.00

T7 Express *lysY/I*q Competent *E. coli*
Highest level of expression control

T7 Express *lysY/I*q Competent *E. coli*
(High Efficiency)
C3013H 20 tubes x 0.05 ml.....$160.00
C3013I 6 tubes x 0.2 ml.....$125.00

T7 Express High Efficiency Sampler
Try each of our four T7 Express strains

T7 Express High Efficiency Sampler
C3009I 8 tubes x 0.2 ml.....$175.00

T7 Express Crystal Competent *E. coli*
For crystallography experiments

T7 Express Crystal Competent *E. coli*
(High Efficiency)
C3022H 20 tubes x 0.05 ml.....$200.00
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Catalogue data sheet of *Escherichia coli* CIP 107305 [Help]

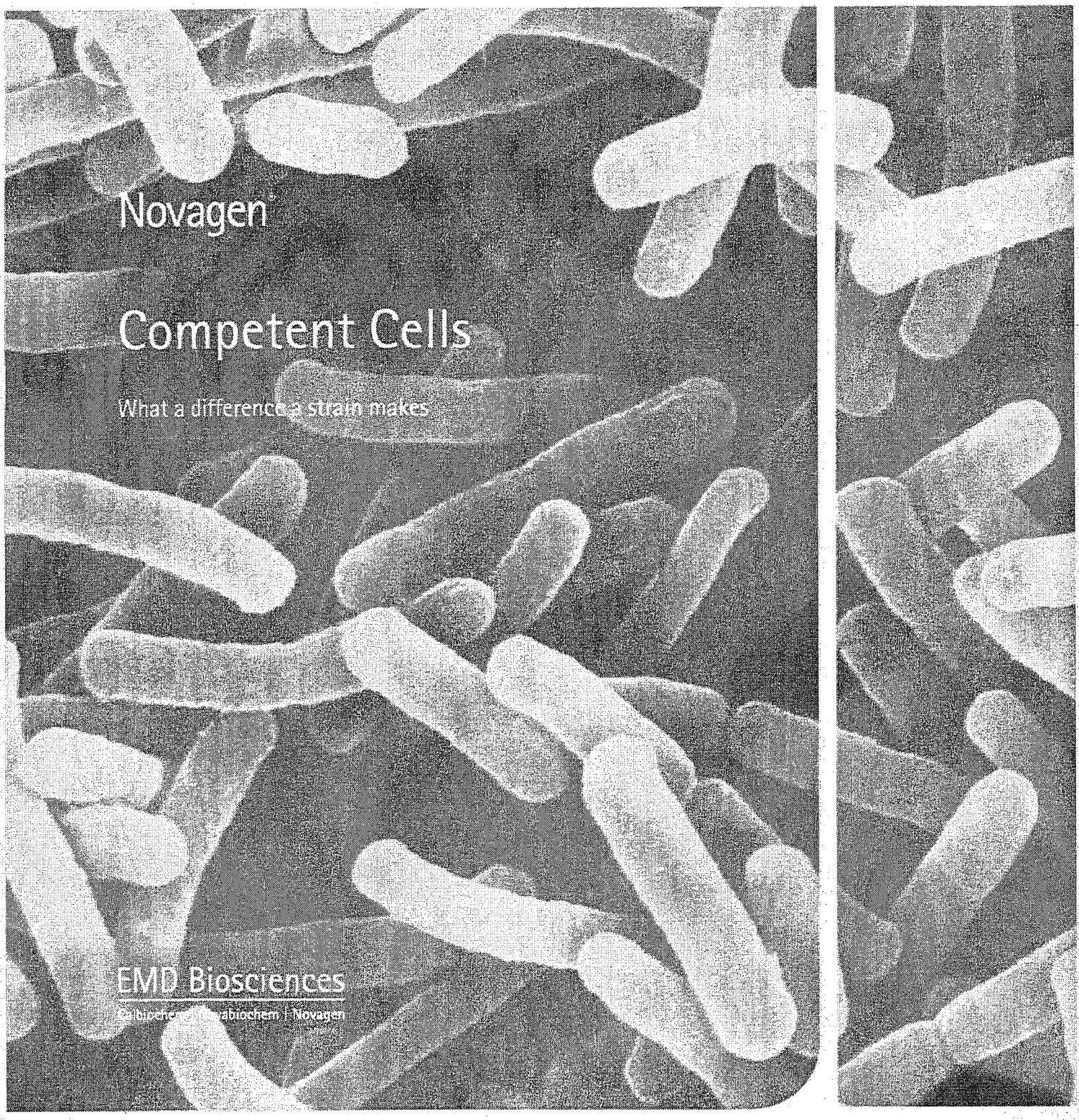
Escherichia coli (Migula 1895) Castellani and Chalmers 1919
Validation or notification list: 1980, *30*, 296
Pathogenicity group: 2

107305
← 2001, A.P. Pugsley, Inst. Pasteur, Paris, France: strain BL21 (lambda DE3)
Genotype: F- ompT (lon) hsdSB(rB- MB-)
Contains DE3, a lambda prophage carrying the T7 RNA polymerase gene under control of plac
Methods in Enzymology, 1990, *185*, 60-89.
Medium: 72, 30°C. Aerobic.

View the sequences, pictures, associated with this item.

close Print

ATTACHMENT B


Novagen®
Competent Cells
What a difference a strain makes
EMD Biosciences
EMD

Inactive proteins?

Express active folded proteins with disulfide bonds in *E. coli*.

Codon bias?

Express mammalian proteins more efficiently in *E. coli* without tedious codon optimization. Use a bacterial host system that supplies 7 rare codon tRNAs.

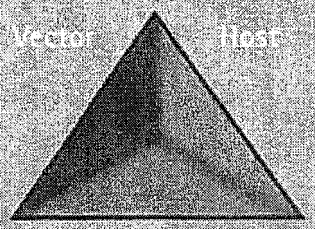
Insoluble protein?

Fine tune your expression levels to avoid aggregation.

What a difference a strain makes!

Novagen competent cells embody the widest selection available for protein expression and offer fundamental strains for cloning applications. We verify the phenotype and purity of each strain and guarantee its transformation efficiency. To meet your needs for maximized yield and activity of target proteins, we offer expression strains that allow stringent control over basal expression levels, enable disulfide bond formation in the cytoplasm, and alleviate codon usage incompatibilities. Chemically competent NovaBlue strains are an excellent choice for routine cloning. For cloning applications that require highest transformation efficiencies, our electrocompetent cell strains have a genotype optimized to construct large, complex libraries. See for yourself what a difference a strain makes!

Features Determining Vector–Host Compatibility



Three factors influence protein expression: the expression vector, host cell, and growth/induction conditions. Changing one or more of these factors can dramatically influence expression levels and target protein solubility.

Vector–Host Relationship

Any number of systems may be suitable for expression of analytical amounts of some proteins for screening, yet only one combination of vector, host strain, and culture conditions may work best for other proteins, for activity assays, and for larger-scale production. If you need a high yield of active protein, it is worth testing a matrix of vector, host, and culture conditions to find the optimal result. To do this, it helps to know more about the target protein and also to empirically determine expression optima by using Novagen competent cell sets, Quarters™ Competent Cells and QuarterPack™ Competent Cell Arrays.

Vector–Host Compatibility

You can use Novagen host strains with many different expression vectors, as long as the plasmid replicon and antibiotic-resistance markers are distinct from corresponding elements carried by the host.

Protein Expression Troubleshooting Guide

Symptom	Possible Problem	Solution	Suggested Host
No protein Truncated protein	*E. coli* codon usage (codon bias)	Supply rare tRNAs	Rosetta™ Rosetta 2 Rosetta-gami™ 2 Rosetta-gami B RosettaBlue
Insoluble protein	Reduction of disulfide bonds	Minimize reduction in cytoplasm	Origami™ 2 Rosetta-gami 2 Rosetta-gami B
	Too much expression	Attenuate expression (titrate IPTG)	Tuner™ Rosetta-gami B
No activity	Misfolded protein	Minimize reduction in cytoplasm	Origami™ 2 Rosetta-gami 2 Rosetta-gami B
		Attenuate expression (titrate IPTG)	Tuner Rosetta-gami B
Cell death	Toxic protein	More stringent control over basal expression	pLysS hosts
No colonies	High basal expression	More stringent control over basal expression	pLysS hosts

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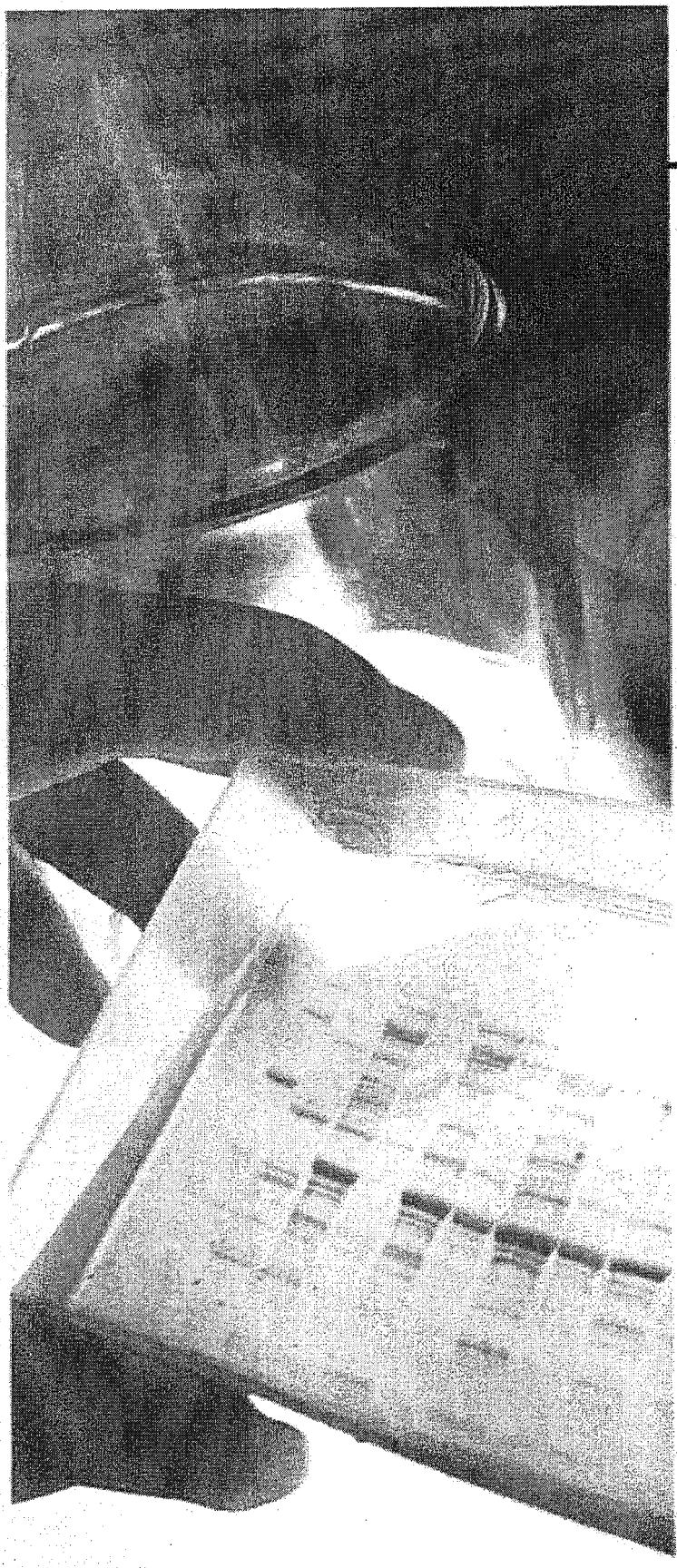


Expression

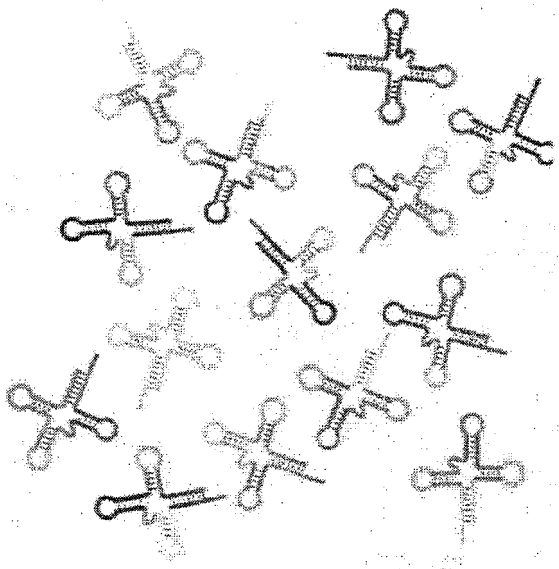
A variety of expression hosts

Expression host strains in many different versions can be used with a variety of protein expression systems. For production of protein from target genes cloned in T7 expression vectors, lysogens of λDE3 carry a chromosomal copy of the T7 RNA polymerase gene under the control of the *lacUV5* promoter. Look for a strain having a pLysS designation; these hosts carry a plasmid that encodes T7 lysozyme, a natural inhibitor of T7 RNA polymerase. Use these strains to suppress basal expression of T7 RNA polymerase before induction, and thereby, stabilize recombinants in pET, pRSF, and pCDF and pCOLA vectors, which encode target proteins that affect cell growth and viability. For expression from *E. coli* promoters such as *tac*, *lac*, *trc*, and p_L, or for T7-based expression by infection with λCE6, versions of these host strains that lack T7 RNA Polymerase also are available.

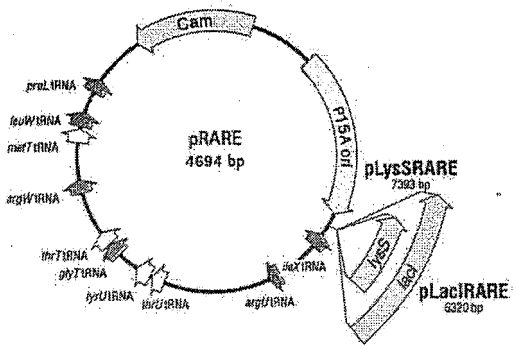
BL21–still the gold standard

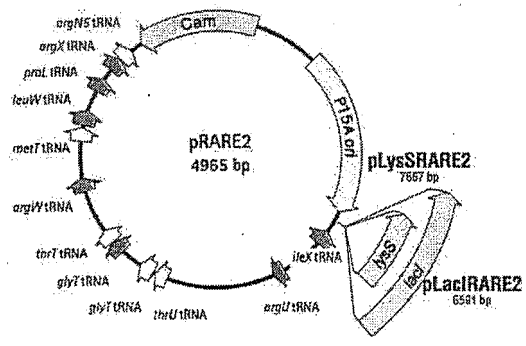
For routine protein expression, **BL21** is an ideal starting point. First commercialized in 1990, the Novagen BL21 strain has remained the gold standard among expression hosts ever since. BL21 and its derivatives are deficient in both *lon* and *ompT* proteases (1). The parental strain, **B834** is a methionine auxotroph that allows high specific activity labeling of target proteins with ^{35}S-methionine or selenomethionine for crystallography studies (2). **BLR**, the *recA*⁻ derivative of BL21, may help stabilize target plasmids containing repetitive sequences or whose products may cause the loss of the DE3 prophage (3, 4). **Tuner™**, the *lacZY* deletion mutant of BL21, enables adjustable levels of protein expression throughout all cells in a culture. Its *lac* permease (*lacY*) mutation allows uniform entry of IPTG into all cells in the population, which produces a concentration-dependent, homogeneous level of induction. By adjusting the IPTG concentration, expression can be regulated from very low levels up to robust, fully induced levels commonly associated with pET hosts. Lower level expression may enhance the solubility and activity of difficult target proteins.

Seven rare tRNAs



Rosetta™ and **Rosetta 2** host strains are BL21 derivatives designed to enhance the expression of eukaryotic proteins that contain codons rarely used in *E. coli*. By supplying these rare tRNAs, the Rosetta strains provide for "universal" translation, which would otherwise be limited by the codon usage of *E. coli*. The original Rosetta strains carry the pRARE plasmid (5) and supply tRNAs for the codons AUA, AGG, AGA, CUA, CCC, and GGA on a chloramphenicol-resistant plasmid. Rosetta 2 strains carry the pRARE2 plasmid and supply a seventh rare tRNA for CGG. In the pLysS and pLacI derivatives of these strains, the rare tRNA genes are present on the same plasmids that carry the T7 lysozyme and *lac* repressor genes, respectively.





Rare codons in *E. coli*

Amino acid	Codon	Fraction in all genes[a]	Fraction in Class II[b]
Arg	AGG	0.022	0.003
Arg	AGA	0.039	0.006
Arg	CGG	0.098	0.008
Arg	CGA	0.065	0.011
Arg	CGU	0.379	0.041
Arg	CGC	0.398	0.370
Gly	GGG	0.151	0.044
Gly	GGA	0.109	0.020
Gly	GGU	0.337	0.508
Gly	GGC	0.403	0.428
Ile	AUA	0.073	0.006
Ile	AUU	0.507	0.235
Ile	AUC	0.420	0.649
Leu	UUG	0.129	0.034
Leu	UUA	0.123	0.055
Leu	CUG	0.496	0.767
Leu	CUU	0.101	0.056
Leu	CUC	0.104	0.080
Pro	CCG	0.525	0.758
Pro	CCA	0.191	0.153
Pro	CCU	0.160	0.112
Pro	CCC	0.124	0.016

REFERENCES

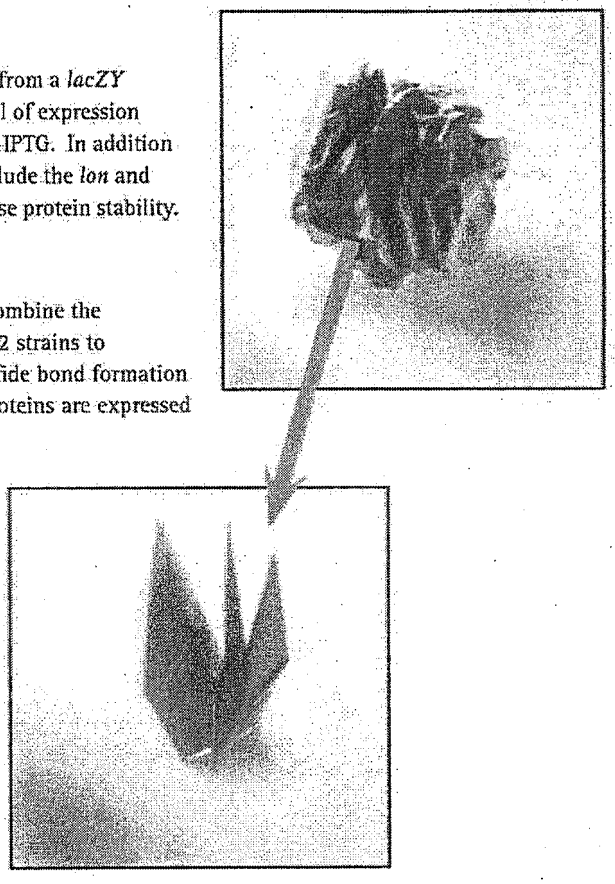
1. Phillips, T. A., Van Bogelen, R. A., and Neidhardt, F. C. (1984) *J. Bacteriol.* 159, 283–287.
2. Leahy, D. J., Hendrickson, W. A., Aukhil, I., and Erickson, H. P. (1992) *Science* 258, 987–991.
3. A. Roca (University of Wisconsin-Madison), personal communication.
4. Studier, F. W. (1991) *J. Mol. Biol.* 219, 37–44.
5. Novy, R., Drott, D., Yaeger, K., and Mierendorf, R. (2001) *inNovations* 12, 1–3.
6. Nakamura, Y., Gojobori, T., and Ikemura, T. (2000) *Nucl. Acids Res.* 29, 292.
7. Henaut, A. and Danchin, A. (1996) in *Escherichia coli and Salmonella typhimurium Cellular and Molecular Biology*, Vol. 2, (Neidhardt, F., Curtiss III, R., Ingraham, J., Lin, E., Low, B., Magasanik, B., Reznikoff, W., Riley, M., Schaechter, M., and Umbarger, H., eds), pp. 2047–2066, American Society for Microbiology, Washington, DC.

Enhanced disulfide bond formation

Origami™ 2 host strains are K-12 derivatives that have mutations in both the thioredoxin reductase (*trxB*) and glutathione reductase (*gor*) genes, which greatly enhance disulfide bond formation in the cytoplasm. Unlike the original Origami strains, the Origami 2 strains are kanamycin sensitive; like the original strains, the *gor* mutation is still selected for by tetracycline. To reduce the possibility of disulfide bond formation between molecules, hosts containing the *trxB/gor* mutation are recommended only for the expression of proteins that require disulfide bond formation for proper folding.

Origami B host strains are derived from a *lacZY* mutant of BL21 to enable precise control of expression levels by adjusting the concentration of IPTG. In addition to *trxB/gor* mutantions these strains include the *lon* and *ompT* deficiencies of BL21 which increase protein stability.

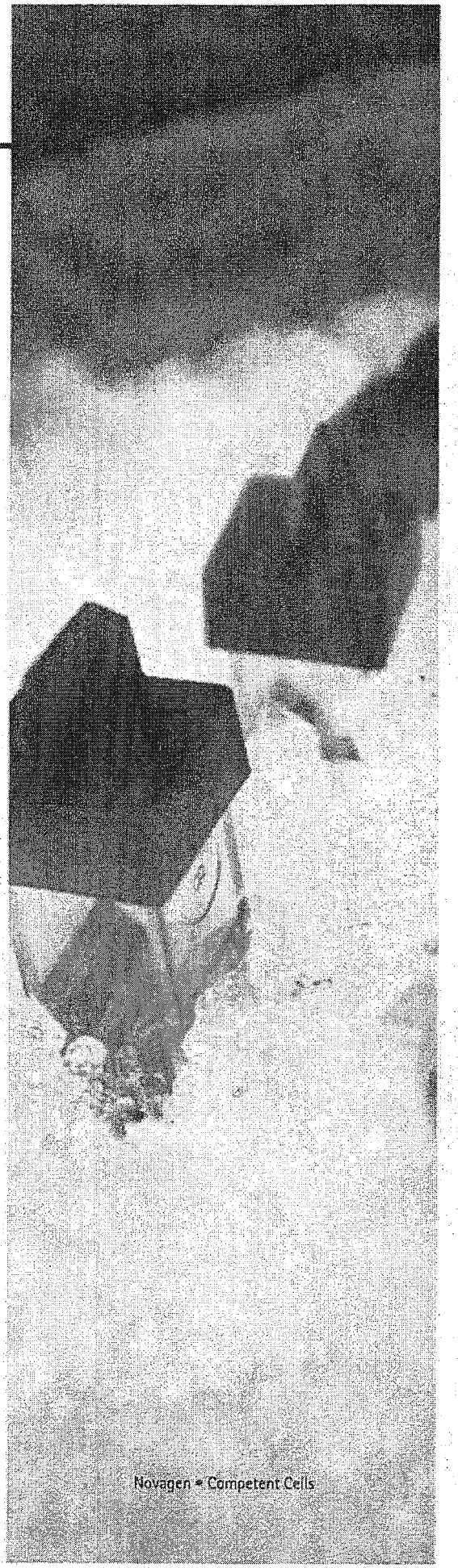
Rosetta-gami™ 2 host strains combine the advantages of Rosetta™ 2 and Origami 2 strains to alleviate codon bias and enhance disulfide bond formation in the cytoplasm when heterologous proteins are expressed in *E. coli*. These *trxB/gor* mutants are compatible with kanamycin-resistant vectors, and carry the chloramphenicol-resistant pRARE2 plasmid, which supplies seven rare tRNAs.



Cloning

High-efficiency electrocompetent cells

NovaXG and NovaXGF' Zappers™ Electrocompetent Cells combine favorable genotype with high transformation efficiency for the most demanding cloning applications. NovaXG features deletion of genes involved in restriction of methylated DNA, [Δ(*mcrC-mrr*)], and *recA endA* mutations, which facilitate high yields of excellent quality plasmid DNA. The *lacZ* Ω fragment is expressed from the chromosome and allows blue/white screening for recombinants by *lacZ* α-complementation with appropriate vectors. NovaXGF' cells have the same genotype as NovaXG, but harbor an F' which confers tetracycline resistance and allows for infection by M13 for ssDNA production. Because the F' carries the *lacI*^{q} repressor gene, addition of IPTG is required for blue/white screening of recombinants in these cells. Both strains are manufactured for high transformation efficiency (> 1 × 10^{9} cfu/µg) by electroporation to deliver a maximum number of transformants, which is especially important when working with limited amounts of DNA or when constructing large or complex libraries. The cells are packaged in a convenient two transformations per tube format to minimize thawing of excess cells.



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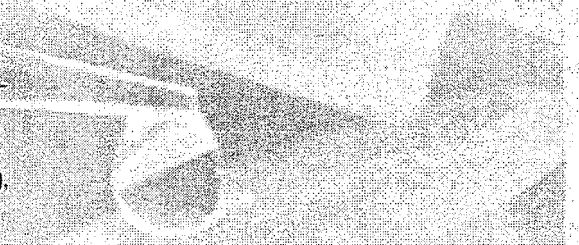

NovaBlue Competent Cells are designed for ultimate convenience and reliability in plasmid transformation. NovaBlue is a K-12 strain ideally suited as an initial cloning host due to its high transformation efficiency, blue/white screening capability (with appropriate plasmids), and *recA endA* mutations, which result in high yields of excellent-quality plasmid DNA. The cells are grown and made chemically competent by an optimized procedure. Select **NovaBlue GigaSingles™** for applications requiring higher transformation efficiencies or **NovaBlue Singles™** for more routine cloning applications. **Veggie™ NovaBlue Singles** are maintained and manufactured with media and reagents derived from nonanimal sources, making these cells ideally suited for applications in which animal-free materials are desired. **NovaBlue T1®** have the same features as NovaBlue Singles, with the added benefit of being resistant to T1 and T5 phage.

NovaBlue Competent Cells format	Transformation efficiency (cfu/µg)	Reaction Size	Application
GigaSingles™	> 1 x 10^9	50 µl	High-efficiency cloning
Singles™	> 1.5 x 10^8	50 µl	Routine cloning
Veggie™	> 1.5 x 10^8	50 µl	Applications requiring nonanimal-derived materials Routine cloning
HT96™	> 1.0 x 10^8	96 x 20 µl	High-throughput cloning
T1®	> 1.5 x 10^8	50 µl	T1/T5 Phage resistant Routine cloning

Technical Support
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E-mail novagen@emdbiosciences.com

Overnight Express

High-level protein expression without the need to monitor cell growth

Two Overnight Express™ Autoinduction Systems are available, both featuring high-level protein production in the pET and other IPTG-inducible bacterial expression systems without the need to monitor cell growth or add an inducer. Cell mass and target protein yield are often increased several-fold as compared with conventional protocols using induction with IPTG.

Overnight Express Protocol
- Prepare medium
- Inoculate with a single colony
- Incubate 8 to 24 hours
- Harvest target protein

Features
- High cell densities and protein expression levels
- No need to monitor cell growth rate or add inducer
- Ideal for pET Expression System or other IPTG-inducible bacterial systems
- Induction of numerous expression clones simultaneously
- Compatible with cultures grown in flasks, culture tubes, and deep-well plates
- Minimal sample handling
- Minimal lot-to-lot variability

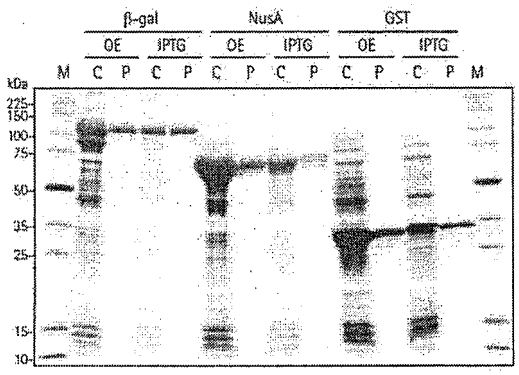
Additional features of Overnight Express Autoinduction System 2
- Complete chemically defined medium
- Ideal for selenomethionine labeling of proteins to be crystallized for x-ray diffraction studies

Product	Size	Cat. No.	Price
Overnight Express™ Autoinduction System 1	1 kit*	71300-3	$55
	1 kit*	71300-4	$220
Overnight Express Autoinduction System 2	1 kit†	71366-3	$98
	1 kit†	71366-4	$392

*includes enough reagents to induce 1 liter
†includes enough reagents to induce 5 liters

Available separately:

Product	Size	Cat. No.	Price
L-Selenomethionine	250 mg	561505	$121
	1 g		$286



Lane	Sample
M	Perfect Protein™ Markers, 10–225 kDa
OE	Overnight Express autoinduction
IPTG	IPTG induction
C	Crude protein extract (equal volumes loaded)
P	Purified target protein (equal protein mass loaded)

Expression and purification of target proteins from cultures induced with Overnight Express versus IPTG

pET recombinants encoding β-gal, NusA, and GST His•Tag® fusion proteins were transformed into BL21(DE3). Protein expression was induced in parallel cultures either by Overnight Express System 1 or 1 mM IPTG. Cells were harvested by centrifugation and extracted with BugBuster® HT Protein Extraction Reagent plus rLysozyme™ Solution. Equal volumes (7 µl) of the extract were analyzed by SDS-PAGE (10–20% gradient gel) and Coomassie blue staining (C lanes). The remainder of the extract was used for robotic affinity purification using Ni-NTA His•Bind® Resin. Samples (2 µg) of the purified fraction were loaded on the gels (P lanes).

Antibiotics

Product	Size	Cat. No.	Price
Carbenicillin	5 g	69101-3	$178
	25 g	220551	$37
Chloramphenicol	100 g		$134
	500 g		$484
Kanamycin Sulfate	5 g	420311	$42
	25 g		$160
	10 g	59346	$27
Tetracycline Hydrochloride	25 g		$37
	50 g		$69

Accessory Products

Product	Size	Cat. No.	Price
ColiRollers™ Plating Beads	1 pkg	71013-3	$8
	5 pkg	71013-4	$33
Veggie™ Peptone	500 g	71280-3	$63
Veggie Yeast Extract	500 g	71279-3	$84
HT96 Isothermal Block		71195-3	$181
100 mM IPTG Solution	15 ml	70527-3	$59
X-Gal Solution	3 x 1 ml	71077-3	$59

For more information about these products visit our website at www.emdbiosciences.com

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Optimized packaging

Novagen competent cells are featured in many different packaging formats. In addition to the Standard 0.2-ml volumes in 20- and 50-reaction kit sizes, several strains are available as Singles™ Competent Cells, single-use, 50-µl volumes for extra convenience and efficiency. Quarters™ Competent Cells consist of 24 wells in a 3 × 8–well configuration that makes up one "quarter" section of a 96-well plate. Each well contains 20 µl competent cells. Quarters sections are ideal for high-throughput screening using multiple strain genotypes for optimization of target protein expression. The BL21(DE3) expression strain and the NovaBlue cloning host are available as HT96™ configurations, which contain 20-µl volumes of competent cells per well in an automation-compatible, 96-well format. For other HT96 configurations or other special packaging needs, contact our Bulk Department.

Host Features Determining Vector Compatibility

Host Strain	Extrachromosomal Replicon(s) in Host	Host Drug Resistance(s)
pLysS-containing cells	P15A	Cam
pLacI-containing cells	P15A	Cam
Rosetta™	P15A	Cam
Rosetta 2	P15A	Cam
Origami™ 2	F	Tet + Str*
Rosetta-gami™ 2	P15A + F	Cam + Tet + Str*
Rosetta-gami	P15A + F	Cam + Kan + Tet+ Str*
BL21	none	none
NovaBlue	F	Tet
Origami B	none	Kan + Tet
RosettaBlue™	P15A + F	Cam + Tet
Rosetta-gami B	P15A	Cam + Kan + Tet
Tuner™	none	none
BLR	none	Tet
HMS174	none	Rif

* These strains carry a mutation in ribosomal protein (rspL) conferring resistance to streptomycin; however, streptomycin is not necessary to maintain strain genotype.

Competent Cell Kit Configurations

Kit Component	Standard Kits		Singles™		Quarters	QuarterPack™ Array	HT96®		Electrocompetent Cells	
	0.4 ml	1 ml	11 rxn	22 rxn			1 plate	4 plates	10 rxn	20 rxn
Competent Cells	2 × 0.2 ml	5 × 0.2 ml	11 × 50 µl	22 × 50 µl	24 × 20 µl	4 × (24 × 20 µl)	96 × 20 µl	4 × (96 × 20 µl)	5 × 50 µl	10 × 50 µl
Test Plasmid	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	2 × 10 µl	10 µl	10 µl
SOC Medium	2 × 2 ml	4 × 2 ml	2 × 2 ml	4 × 2 ml	2 × 2 ml	14 ml	14 ml	4 × 14 ml		
8-cap Strip					pkg/12	pkg/12	pkg/12	4 × (pkg/12)		
Reagent Reservoir					1	1	1	4		
HT96 Lids							1	4		

Competent Cell Selection

Protein Expression Strains	Subtype	Singles 11 reactions	Singles 22 reactions	Standard 0.4 ml	Standard 1.0 ml	Quarters™ 24 reactions
Pricing (2004)		$87	$170	$70	$129	$87
B834	(DE3)			69041-3	69041-4	
	(DE3)pLysS			69042-3	69042-4	
BL21*				69449-3	69449-4	71158-3
	(DE3)	70235-3	70235-4	69450-3	69450-4	71159-3
	(DE3)pLysS	70236-3	70236-4	69451-3	69451-4	71160-3
BLR				69052-3	69052-4	
	(DE3)			69053-3	69053-4	
	(DE3)pLysS			69956-3	69956-4	
HMS174				69452-3	69452-4	
	(DE3)			69453-3	69453-4	
	(DE3)pLysS			69454-3	69454-4	
Origami™ 2				71344-3	71344-4	
	(DE3)	71408-3	71408-4	71345-3	71345-4	
	(DE3)pLysS	71409-3	71409-4	71346-3	71346-4	
Origami B				70836-3	70836-4	71162-3
	(DE3)			70837-3	70837-4	71163-3
	(DE3)pLysS			70839-3	70839-4	71164-3
Rosetta™				70953-3	70953-4	71166-3
	(DE3)			70954-3	70954-4	71167-3
	(DE3)pLysS			70956-3	70956-4	71168-3
Rosetta 2				71402-3	71402-4	
	(DE3)	71400-3	71400-4	71397-3	71397-4	
	(DE3)pLysS	71401-3	71401-4	71403-3	71403-4	
RosettaBlue™				71058-3	71058-4	
	(DE3)			71059-3	71059-4	
	(DE3)pLysS			71034-3	71034-4	
Rosetta-gami™ 2				71350-3	71350-4	
	(DE3)			71351-3	71351-4	
	(DE3)pLysS			71352-3	71352-4	
Rosetta-gami B				71136-3	71135-4	71170-3
	(DE3)			71136-3	71136-4	71171-3
	(DE3)pLysS			71137-3	71137-4	71172-3
Tuner™				70622-3	70622-4	
	(DE3)			70623-3	70623-4	
	(DE3)pLysS			70624-3	70624-4	

*Also available – HT96, 1 Plate: 71012-3 $306; HT96, 4 Plates: 71012-4 $1146

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Cloning Strain	Singles 11 rxn	Singles 22 rxn	GigaSingles™ 11 rxn	GigaSingles 22 rxn	Standard 0.4 ml	Standard 1.0 ml	HT96 1 plate	HT96 4 plates	Electrocompetent 10 rxn	Electrocompetent 20 rxn
Pricing (2004)	$87	$170	$105	$204	$70	$129	$306	$1146	$95	$171
NovaBlue	70181-3	70181-4	71227-3	71227-4	69825-3	69825-4	71011-3	71011-4		
NovaXG									71315-3	71316-4
NovaXGF									71317-3	71317-4
NovaBlue T1R	71318-3	71318-4								
Veggie™ NovaBlue	71251-3	71251-4								

†$115 for Veggie NovaBlue Singles 11 rxn; ‡$227 for Veggie NovaBlue Singles 22 rxn

For more information or to order Novagen products, contact EMD Biosciences.

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